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Influence of lactic acid fermentation on the microbiological parameters, biogenic amines, and volatile compounds of bovine colostrum

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ABSTRACT

In this study we hypothesized that the relations between the bovine colostrum (BC) microbiota, biogenic amine (BA) as well as volatile compound (VC) profiles can lead to new deeper insights concerning the BC changes during the biological preservation. To implement such an aim, BC samples were collected from 5 farms located in Lithuania and fermented with *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* strains. Nonfermented and fermented BC were subjected to microbiological [lactic acid bacteria (LAB), *Escherichia coli*, and total bacteria (TBC), total Enterobacteriaceae (TEC) and total mold and yeast (M-Y) viable counts] and physicochemical (pH, color coordinates, BA content and VC profile) parameters evaluation, and the relationship between the tested parameters were also further analyzed. In comparison pH and dry matter (DM) of nonfermented samples, significant differences were not found, and pH of BC was, on average, 6.30, and DM, on average, 27.5%. The pH of fermented samples decreased, on average, until 4.40 in *Lp. plantarum* fermented group, and, on average, until 4.37 in *Lc. paracasei* fermented group. Comparing color characteristics among nonfermented BC groups, significant differences between lightness (L*) and yellowness (b*) were not detected, however, the origin (i.e., agricultural company), LAB strain used for fermentation and the interaction between these factors were statistically significant on BC redness (a*) coordinate. The microbial contamination among all the tested BC groups was similar. However, different LAB strains used for BC fermentation showed different effects toward the microbial contamination reduction, and specifically *Lc. paracasei* was more effective than *Lp. plantarum* strain. Predominant BA in BC were putrescine and cadaverine. The main VC in nonfermented and fermented BC were decane, 2-ethyl-1-hexanol, dodecane, 1,3-di-tert-butylbenzene, 3,6-dimethyldecane and tetradecane. Moreover, this study showed worrying trends with respect to the frozen colostrum storage, because most of the dominant VC in BC were contaminants from the packaging material. Additionally, significant correlations between separate VC and microbial contamination were obtained. Finally, these experimental results showed that the separate VC in BC can be an important marker for biological as well as chemical contamination of BC. Also, it should be pointed out that despite the fermentation with LAB is usually described as a safe and natural process with many advantages, control of BA in the end product is necessary.

Key words: bovine colostrum, fermentation, biogenic amines, volatile compounds, quality

INTRODUCTION

For many years bovine colostrum (**BC**) has been known as a valuable multifunctional material not just for newborn calf feeding but also for human consumption. A healthy cow produces, on average, 7.5 L of colostrum per milking (Moore et al., 2009), and this amount usually exceeds the requirements of the newborn calf (Gomes et al., 2021). Therefore, its surplus can be used for the production of nutraceuticals and supplements for human consumption. Because of the BC therapeutic effect, most of the studies are focused on the analysis of the functional compounds of BC: immunoglobulins, lactoferrin, fatty acid constituents, and so on (Playford and Weiser, 2021). However, despite

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the fact that nowadays the number of reports about the possible preservation of bovine colostrum is increasing, reports including data about biogenic amines (**BA**) formation and changes in BC volatile compounds (**VC**) are scarce, so far. Finally, the problem associated with BC preservation is still relevant. It was reported that 90% of Irish and 89% of North America dairy producers routinely store BC (Cummins et al., 2017). Microorganisms in BC double every 30 min at 21°C (Stewart et al., 2005) and, for this reason, BC in farms is stored in freezers (Haggerty et al., 2021). It was reported that more than 40% of the tested BC samples exceeded in total bacteria viable count (**TBC**) thresholds [>100 000 colony-forming units (cfu)/mL] (Fecteau et al., 2002; Morrill et al., 2012; Phipps et al., 2016), and, on average, 90% of pooled colostrum samples were highly contaminated (Denholm et al., 2017). Microbial contamination may reduce functionality of the BC, chiefly coliform species impair IgG absorption (Gelsinger et al., 2015), pathogens physically block absorption channels of the immunoglobulin molecules (Corley et al., 1977; James et al., 1981; Staley and Bush, 1985) and may damage intestinal cells (Denholm, 2022). In addition, microbial pathogen contamination can cause diseases in calves (Denholm, 2022). Also, some of the opportunistic pathogenic microorganisms (mainly enterobacteria) possess decarboxylase activities, which can lead to the formation of BA. The latter compounds are formed from amino acids by decarboxylation, or by amination and transamination of aldehydes and ketones (Nout, 2014). Some BA have potentially detrimental effects to animal (Driehuis et al., 2018) and human health (Lappa et al., 2022).

Usually, BC preservation is attained by the addition of chemical preservatives, by freezing or by fermentation (Denholm, 2022). Fermentation with selected lactic acid bacteria (**LAB**) strains possessing antimicrobial properties may be a good alternative to low temperature storage or chemical preservation. In this research study, the employed LAB strains (*Lactiplantibacillus plantarum* LUHS135 and *Lacticaseibacillus paracasei* LUHS244) previously showed desirable antimicrobial properties to inhibit pathogenic opportunistic bacteria strains and fungi, which has been multiplied in selective medias (Bartkiene et al., 2020a), as well as, these LAB strains showed characteristics to reduce biological contamination of colostrum samples (Bartkiene et al., 2020b). However, it should be underlined that in addition to the desirable properties of LAB to reduce BC pH and to increase biosafety, they can also form BA. Taking into consideration that LAB metabolic activities are dependent on the specific substrates, the BC collected from different origins (agricultural companies) may show different parameters after fermentation, because of the different microconstituents of bovine colostrum—including free amino acids, which are precursors of BA. Additionally, distinct strains used for BC fermentation can lead to different volatile compound (**VC**) formation. Several studies reported about the contribution of LAB strains on the sensory properties of traditional dairy products (Medeiros et al., 2016; Tian et al., 2019; Madhubasani et al., 2020; Mehrinejad Choobari et al., 2021). However, the data about BC VC and their changes during the fermentation process are scarce.

In this study we hypothesized that the relationship between microbiological, BA and VC profiles of BC samples can lead to new deeper insights and knowledge concerning the BC changes throughout the biological preservation. To implement such an aim, BC samples were collected from 5 farms located in Lithuania and fermented with *Lp. plantarum* and *Lc. paracasei* strains. Nonfermented and fermented BC samples were subjected to microbiological (LAB, *Escherichia coli*, total bacteria, total enterobacteria, and mold/yeast viable counts) and physicochemical (pH, color coordinates, BA content, and VC profile) parameters evaluation, and the relationships between the tested parameters were analyzed.

MATERIALS AND METHODS

Because no invasive procedures were performed, this study did not require institutional animal care and use approval.

Materials

The BC samples were collected from the different regions of Lithuania from 5 farms keeping (confined keeping system) Holstein dairy cows during 2022, from the beginning of March until the end of May: (1) from Dauksiai city, (2) from Sauselio village, (3) from Geluvos village, (4) from Paliepiu village, and (5) from Alksnupiu village.

At agricultural companies, BC was collected within 2 h of calf delivery and frozen at −18°C before use. For experiment, colostrum samples were collected once from one farm. Colostrum kept at −18°C in plastic bags (1–5 L volume) is the usual practice at agricultural companies in Lithuania. Usually, in farms, BC is kept frozen for no longer than 1 wk, before the use. Samples of BC, before transportation to the laboratory, were kept frozen for 5 d.

Lactiplantibacillus plantarum LUHS135 and *Lacticaseibacillus paracasei* LUHS244 strains, for BC fermentation, were selected according to their good antibacterial and antifungal properties (Bartkiene et al., 2020a).

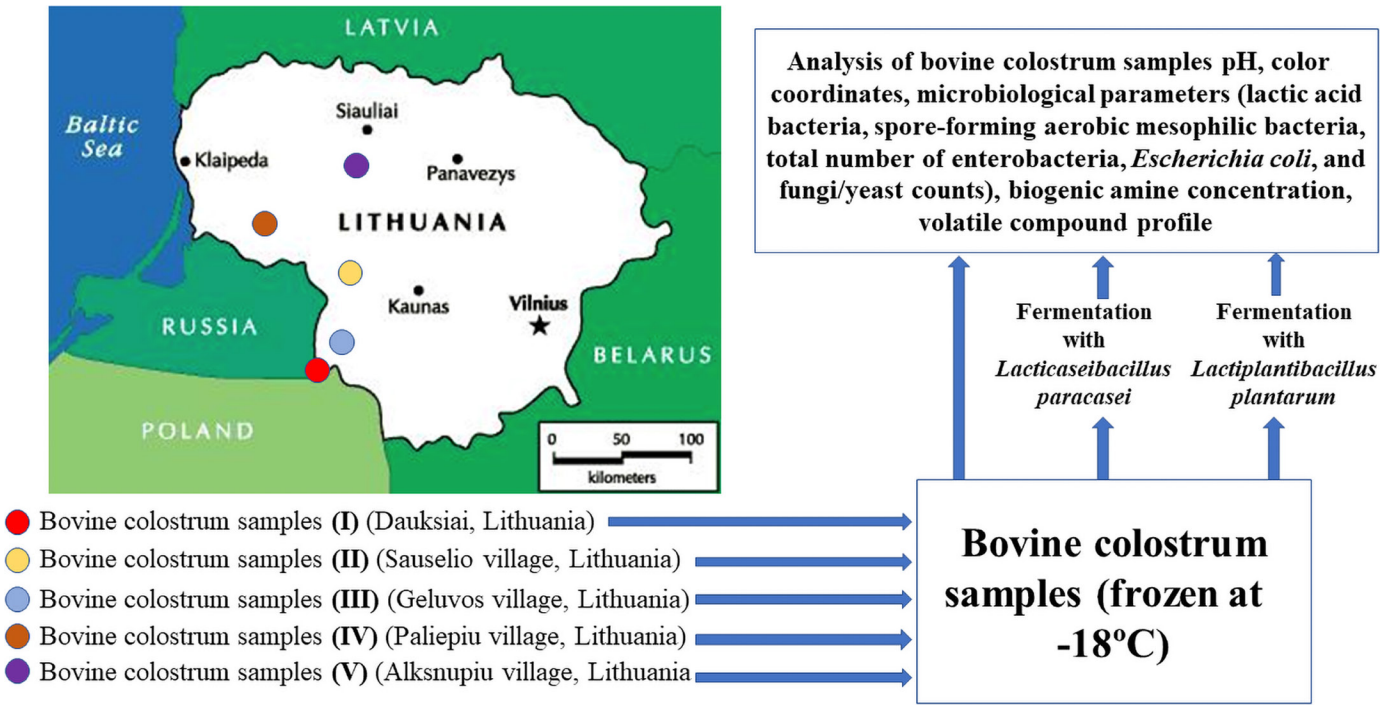

Figure 1. Schematic representation of the experimental design, in which BC samples were collected from different regions of Lithuania from 5 farms keeping Holstein dairy cows.

Before the experiment, *Lp. plantarum* and *Lc. paracasei* were stored at −80°C in a Microbank system (Pro-Lab Diagnostics, UK) and grown in de Man, Rogosa, Sharpe (**MRS**) broth (CM 0359, Oxoid, Hampshire, UK) at 30°C for 48 h before use.

Fermentation of BC

Before BC fermentation, *Lp. plantarum* and *Lc. paracasei* strains were incubated and multiplied (individually) in MRS medium (Biolife, Milan, Italy) at 30°C for 48 h. Three milliliters of MRS broth, in which the 2 LAB strains were individually grown (average LAB cell concentration of 9.2 $\log_{10}$ cfu/mL), was inoculated into 100 mL of defrosted (at 24 ± 2°C for 12 h) BC, followed by fermentation in a CO_2 incubator (Memmert GmbH + Co. KG, Schwabach, Germany) for 24 h at 30°C.

In total, 15 sample groups were analyzed, such as nonfermented BC samples: (1), (2), (3), (4), and (5); with *Lp. plantarum* fermented BC samples: (1) Lpl, (2) Lpl, (3) Lpl, (4) Lpl, and (5) Lpl; and with *Lc. paracasei* fermented BC samples: (1) Lpa, (2) Lpa, (3) Lpa, (4) Lpa, and (5) Lpa. The experimental design is schematized in Figure 1.

The number of BC samples from each farm was collected, taking into account its total number of cows, chiefly: 25 samples (from 500 keeping cows) from I farm; 6 samples (from 120 keeping cows) from II farm; 7 samples (from 159 keeping cows) from III farm; 5 samples (from 131 keeping cows) from IV farm; and 70 samples (from 1,403 keeping cows) from V farm.

Determination of pH, Color Coordinates (L*, a*, and b*), and DM in Bovine Colostrum

The pH of BC was evaluated with a pH meter (Inolab 3, Hanna Instruments, Venet, Italy) by inserting the pH electrode into the samples. The color coordinates of the BC were evaluated on the samples surface using the International Commission on Illumination (**CIE**) L*a*b* color space system (CromaMeter CR-400, Konica Minolta, Marunouchi, Tokyo Japan), where L*, a*, and b* represent the lightness, yellowness, and redness coordinates, respectively. The DM (%) of BC samples was measured with a refractometer Pal-3 (Atago, Japan).

Evaluation of Microbiological Parameters in BC

Nonfermented and fermented BC samples were subjected to microbiological analyses, such as total LAB, *Escherichia coli*, total bacteria (**TBC**), total Enterobacteriaceae (**TEC**), and total mold and yeast (**M-Y**) viable counts. For the evaluation of LAB viable counts, 10 g of BC was homogenized with Ultra-Turrax (IKA Labortechnik, Staufen, Germany) with 90 mL of

saline solution (9 g/L NaCl aqueous solution). Serial decimal dilutions from 10^{-4} to 10^{-8} with saline solution were used for sample preparation. Sterile MRS agar (CM0361, Oxoid) of 5 mm thickness on Petri dishes was used for bacterial growth. Petri dishes were separately inoculated with the sample suspension using surface spread-plate technique and were incubated under anaerobic conditions at 30°C for 72 h. The TBC was determined on plate count agar and were incubated under aerobic conditions at 32°C for 24 to 48 h (CM0325, Oxoid, UK). MacConkey agar (Oxoid Ltd., Basingstoke, United Kingdom) and Tryptone Bile X-glucuronide agar (Oxoid Ltd., Basingstoke, United Kingdom) were used for the determination of the total number of Enterobacteriaceae and *E. coli* (anaerobic conditions at 35–37°C for 18–24 h), respectively. Mold and yeasts were determined on chloramphenicol agar (CM0549, Oxoid, UK) after incubation at 25 ± 2°C for 5 d under aerobiosis. The number of viable microorganisms was counted in the dilutions containing between 30 and 300 colonies and expressed as $\log_{10}$ of colony-forming units per milliliter (cfu/mL).

Qualitative and Quantitative Analysis of BA in Bovine Colostrum

The extraction and determination of BA in nonfermented and fermented BC samples followed the procedures developed by Ben-Gigirey et al. (1999) with some modifications as described by Bartkiene et al. (2020b). In 20 mL of deionized water, stock solutions for each BA (1 mg/mL), including the internal standard 1.7-diamino-heptane, were produced. In short, 10 mL of perchloric acid (0.4 mol/L) were used 2 times to extract 5 g of the BC.

Derivatization of sample extracts and standards was performed with a dansyl chloride solution in acetonitrile (10 mg/mL). Varian ProStar HPLC system (Varian Corp., Palo Alto, CA) equipped with ProStar 325 UV/VIS Detector and Galaxy software (Agilent, Santa Clara, CA) was employed. A Discovery HS C18 column 150 mm length × 4.6 mm-ϕ, 5 μm-ϕ particle size; SupelcoTM Analytical, Bellefonte, Pennsylvania) was used for separation. Ammonium acetate 0.1 mol/L (solvent A) and acetonitrile (solvent B) were used as mobile phases at constant flow rate of 0.8 mL/min. The detection limit of BA was 0.1 mg/kg. The BA were identified based on their retention times in comparison to their corresponding standards.

Qualitative and Quantitative Analysis of VC in BC

The VC of BC samples were determined by GC-MS. A solid-phase microextraction (**SPME**) device with Stableflex fiber coated with a 50 μm PDMS-DVB-Carboxen layer (Supelco) was used for analysis. For headspace extraction, 1 g of sample, 50 μL of 0.93 mg/mL valeric acid solution (internal standard) and 10 mL of phosphate buffer (1 M NaH_2PO_4 solution adjusted with 5% phosphoric acid solution to pH = 3) were transferred to the 20 mL extraction vial, sealed with a polytetrafluoroethylene septum and incubated at 60°C for 15 min before exposing the fiber in the headspace. The fiber was exposed to the headspace of the vial for 10 min and desorbed in an injector liner for 2 min (splitless injection mode). Prepared samples were analyzed with a GCMS-QP2010 (Shimadzu, Japan) gas chromatograph with mass spectrometer. The following conditions were used for analysis: injector temperature 250°C, ion source temperature 220°C, interface temperature 260°C. Helium was used as carrier gas at 0.65 mL/min flow rate. A Rxi-5MS capillary column (0.25-mm ID, 0.25-μm film thickness, 30-m length, Restek) was used for analysis. The temperature gradient was programmed from a start at 40°C (3 min hold) to 220°C (5°C/min) up to 310°C (15°C/min; 6 min hold). The VC were identified according to mass spectrum libraries (NIST11, NIST11S, FFNSC2). To remove false-positive results, retention index matching was used. The retention indices were determined by analyzing mixture of alkanes (C8-C20).

Statistical Analysis

The results were expressed as independent mean value ± standard error of the BC samples collected in each agricultural company. As previously mentioned, taking into consideration that different farms are keeping different number of animals, BC was collected from, on average, 5% of the dairy cows so as to get representative samplings. All samples from each farm were analyzed separately. The analyses of independent samples were repeated in triplicate. The number of viable microorganisms in BC samples was expressed as $\log_{10}$ of colony-forming units per milliliter. To evaluate the effects of different farms, different LAB strains used for fermentation and the interaction between these factors (independent variables) on the BC parameters (dependent variables or observations), data were analyzed by multivariate ANOVA (V22.0, IBM SPSS Statistics, Chicago, IL; 2013). Also, Pearson correlations were calculated between the parameters of BC, and the strength of correlation was interpreted according to Evans (Evans, 1996). Paired-samples t-test was used to evaluate significant differences between BC sample groups. The results were recognized as statistically significant at $P \leq 0.05$.

Table 1. Average values and SE of bovine colostrum pH, color coordinates (L*, a*, and b*), and DM[1]

Sample group no.[2]	pH	Color coordinates, NBS[3]			DM, %
		L*	a*	b*	
Control					
(1)	6.41 ± 0.34[a]	91.9 ± 5.0[a]	2.85 ± 0.27[b]	35.6 ± 1.5[a]	27.2 ± 2.3
(2)	6.22 ± 0.39[a]	97.1 ± 8.1[a]	2.81 ± 0.17[b]	37.6 ± 3.2[a]	26.6 ± 1.3
(3)	6.32 ± 0.42[a]	96.3 ± 4.1[a]	2.77 ± 0.13[b]	37.7 ± 3.0[a]	26.8 ± 2.0
(4)	6.32 ± 0.35[a]	88.2 ± 8.7[a]	2.26 ± 0.20[a]	34.6 ± 2.2[a]	27.1 ± 0.9
(5)	6.25 ± 0.32[a]	92.3 ± 6.9[a]	2.38 ± 0.21[a]	35.1 ± 2.4[a]	29.8 ± 1.6
LUHS135					
(1)	4.67 ± 0.28[a]	89.1 ± 3.5[ab]	0.760 ± 0.070[d]	31.8 ± 1.7[a]	27.6 ± 1.80
(2)	4.52 ± 0.35[a]	83.8 ± 6.5[ab]	0.200 ± 0.010[a]	28.8 ± 1.9[a]	27.1 ± 2.57
(3)	4.22 ± 0.31[a]	94.2 ± 5.2b	0.710 ± 0.050[cd]	29.2 ± 2.4[a]	27.2 ± 1.82
(4)	4.40 ± 0.37[a]	84.6 ± 6.1[ab]	0.290 ± 0.010[b]	31.4 ± 2.2[a]	27.6 ± 2.33
(5)	4.18 ± 0.32[a]	84.8 ± 3.9[a]	0.670 ± 0.020[c]	29.3 ± 1.7[a]	29.4 ± 2.55
LUHS244					
(1)	4.61 ± 0.27[a]	89.5 ± 8.5[ab]	0.960 ± 0.070[c]	31.2 ± 3.0[a]	27.5 ± 1.8
(2)	4.58 ± 0.44[a]	85.4 ± 4.7[ab]	0.820 ± 0.050[b]	28.7 ± 1.5[a]	27.4 ± 0.8
(3)	4.35 ± 0.39[a]	91.4 ± 4.5[b]	0.910 ± 0.040[bc]	27.7 ± 2.0[a]	27.3 ± 1.0
(4)	4.21 ± 0.30[a]	82.9 ± 2.5[a]	0.420 ± 0.020[a]	30.2 ± 0.9[a]	27.5 ± 2.4
(5)	4.10 ± 0.30[a]	86.1 ± 2.9[ab]	0.890 ± 0.060[bc]	30.2 ± 2.1[a]	29.8 ± 1.7

[a–d]Mean values between different samples in group (groups: nonfermented, fermented with LUHS135, and fermented with LUHS244) within a column with different letters are significantly different ($P \leq 0.05$).

[1]Paired-samples *t*-test was used to evaluate significant differences between groups of bovine colostrum samples.

[2](1) = colostrum from Dauksiai, Lithuania, (2) = colostrum from Sauselio village, Lithuania, (3) = colostrum from Geluvos village, Lithuania, (4) = colostrum from Paliepiu village, Lithuania, (5) = colostrum from Alksnupiu village, Lithuania; LUHS135 = fermented with *Lactiplantibacillus plantarum*; LUHS244 = fermented with *Lacticaseibacillus paracasei*.

[3]NBS = National Bureau of Standards units; L* = lightness; a* = redness or b* = yellowness.

RESULTS

Influence of Fermentation on BC pH, Color Coordinates (L, a*, and b*), and DM*

The average values of BC pH, color coordinates, and DM collected in different agricultural companies are given in Table 1. No significant differences were observed in pH and DM of nonfermented samples, and the average values were 6.30 for pH, and 27.5% for DM. In comparison nonfermented and fermented BC samples, in the latter pH average values decreased to 4.40 when fermented with *Lp. plantarum*, and to 4.37 when fermented with *Lc. paracasei*. Multivariate ANOVA showed that the agricultural company and LAB strain used for fermentation were significant factors on BC pH values ($P \leq 0.0001$ and $P = 0.002$, respectively).

Analyzing color characteristics of nonfermented BC sample groups, no significant differences between L* (lightness) and b* (yellowness) coordinates were found (Table 1). However, samples collected from (1), (2), and (3) farms showed, on average, 17.4% higher a* (redness) coordinate values in comparison with samples collected from the remaining farms (i.e., ([4]) and ([5]). Comparing sample groups fermented with *Lp. plantarum*, the lowest and highest L* coordinates were obtained in samples collected from farm (5; 84.8 NBS) and farm (3; 94.2 NBS), respectively. The average L* coordinates in other samples were 85.8 NBS. The lowest a* coordinates were found in samples collected from farm (2; 0.200 NBS), whereas samples collected from farms (4), (5), (3), and (1) showed, on average, by 1.45, 3.35, 3.55, and 3.80 times, respectively, higher a* values. However, no significant differences were found with respect to b* values in sample groups fermented with *Lp. plantarum*. In contrast, comparing sample groups fermented with *Lc. paracasei*, L* values ranged between 82.9 and 91.4 NBS (samples collected from farm (4) and (3), respectively). The lowest a* values were obtained in samples from farm (4), and no significant differences were observed in b* coordinates between the sample groups fermented with *Lc. paracasei* b* (on average, b* value was 29.6 NBS). Comparing fermented sample groups with nonfermented (controls) ones, systematically higher a* coordinate values were found in nonfermented samples, and where lowest a* coordinate values were attained in samples fermented with *Lp. plantarum* strain. In the majority of cases, fermentation reduced b* coordinates on BC, except in samples collected from farm (4) fermented with *Lp. plantarum* strain, as well as in samples collected from farm (1) fermented with *Lc. paracasei* strain (Table 1).

Multivariate ANOVA showed that the origin (farm), LAB strain used for fermentation and the interaction

Table 2. Average values and SE of bovine colostrum microbiological parameters[1]

Sample group no.[2]	LAB[3]	*Escherichia coli*	TBC	TEC	M-Y
Control					
(1)	7.89 ± 0.35[a]	6.82 ± 0.51[a]	7.80 ± 0.56[a]	6.00 ± 0.33[a]	5.80 ± 0.55[a]
(2)	8.01 ± 0.36[a]	6.62 ± 0.24[a]	7.77 ± 0.35[a]	6.00 ± 0.54[a]	5.80 ± 0.31[a]
(3)	8.04 ± 0.80[a]	6.99 ± 0.53[a]	7.83 ± 0.26[a]	6.20 ± 0.23[a]	6.00 ± 0.48[a]
(4)	7.92 ± 0.66[a]	6.74 ± 0.37[a]	8.00 ± 0.41[a]	6.70 ± 0.43[a]	5.70 ± 0.37[a]
(5)	7.92 ± 0.33[a]	6.93 ± 0.48[a]	7.30 ± 0.62[a]	6.20 ± 0.62[a]	6.20 ± 0.27[a]
LUHS135					
(1)	8.37 ± 0.57[a]	2.99 ± 0.20[a]	5.49 ± 0.44[b]	3.00 ± 0.27[a]	1.80 ± 0.06[b]
(2)	8.33 ± 0.27[a]	3.23 ± 0.32[a]	4.87 ± 0.27[ab]	3.40 ± 0.24[a]	2.50 ± 0.12[d]
(3)	8.37 ± 0.28[a]	3.02 ± 0.28[a]	5.02 ± 0.32[ab]	3.40 ± 0.28[a]	1.20 ± 0.04[a]
(4)	8.37 ± 0.79[a]	3.26 ± 0.26[a]	4.80 ± 0.39[ab]	3.10 ± 0.23[a]	2.10 ± 0.08[c]
(5)	8.38 ± 0.39[a]	3.02 ± 0.16[a]	4.60 ± 0.31[a]	3.30 ± 0.16[a]	1.10 ± 0.09[a]
LUHS244					
(1)	8.31 ± 0.26[a]	0.60 ± 0.05[b]	4.18 ± 0.14[d]	1.20 ± 0.07[c]	0.400 ± 0.030[c]
(2)	8.29 ± 0.82[a]	0.48 ± 0.02[a]	1.46 ± 0.11[b]	ND	0.300 ± 0.020[b]
(3)	8.34 ± 0.57[a]	0.92 ± 0.03[c]	1.46 ± 0.07[b]	0.500 ± 0.030[b]	0.800 ± 0.070[d]
(4)	8.33 ± 0.59[a]	0.51 ± 0.03[a]	0.40 ± 0.02[a]	0.100 ± 0.010[a]	0.100 ± 0.020[a]
(5)	8.26 ± 0.43[a]	1.33 ± 0.13[d]	1.70 ± 0.12[c]	0.500 ± 0.040[b]	ND

[a–d]Mean values between different samples in group (groups: nonfermented, fermented with LUHS135, and fermented with LUHS244) within a line with different letters are significantly different ($P \leq 0.05$).

[1]Paired–samples *t*-test was used to evaluate significant differences between groups of bovine colostrum samples.

[2](1) = colostrum from Dauksiai, Lithuania, (2) = colostrum from Sauselio village, Lithuania, (3) = colostrum from Geluvos village, Lithuania, (4) = colostrum from Paliepiu village, Lithuania, (5) = colostrum from Alksnupiu village, Lithuania; LUHS135 = fermented with *Lactiplantibacillus plantarum*; LUHS244 = fermented with *Lacticaseibacillus paracasei*. All values presented in $\log_{10}$ cfu/mL[3].

[3]LAB = lactic acid bacteria; TBC = total bacteria viable counts; TEC = total enterobacteria count, M-Y = mold and yeast.

[4]ND = not detected.

of these factors were statistically significant on BC a* coordinate ($P \leq 0.0001$, $P = 0.002$, and $P \leq 0.001$, respectively).

Influence of Fermentation on BC Microbiological Parameters

Microbiological parameters of bovine colostrum—such as total LAB, *E. coli*, TBC, TEC, total M-Y viable counts—are tabulated in Table 2. Observing the data from nonfermented samples, no significant differences were found in BC collected from different farms. In addition, on average, LAB viable count was 7.96 $\log_{10}$ cfu/mL, *E. coli* viable count was 6.82 $\log_{10}$ cfu/mL, TBC was 7.74 $\log_{10}$ cfu/mL, TEC was 6.22 $\log_{10}$ cfu/mL and M-Y count was 5.9 $\log_{10}$ cfu/mL. However, different trends were established in fermented BC samples. Despite that LAB viable counts in all fermented samples (fermented with *Lp. plantarum* and *Lc. paracasei* strains) were similar (on average, 8.34 $\log_{10}$ cfu/mL), contamination with *E. coli*, TBC, TEC, and M-Y decreased. Evaluating *E. coli* viable counts in nonfermented and fermented samples, greater reduction of *E. coli* was found in samples fermented with *Lc. paracasei*. In samples fermented with *Lp. plantarum* strain, the *E. coli* viable counts were, on average, 3.10 $\log_{10}$ cfu/mL. Similar tendencies were found with TEC and M-Y viable counts, and where the lowest numbers were found in groups fermented with *Lc. paracasei*.

Multivariate ANOVA showed that LAB strain used for fermentation and factor's interaction (LAB strain used for fermentation and farm) were significant on *E. coli* viable counts in BC ($P \leq 0.0001$). Positive weak and moderate correlations were found between colostrum pH and *E. coli* viable counts, TBC, TEC, and M-Y viable counts ($r = 0.521$, $P \leq 0.0001$; $r = 0.417$, $P = 0.004$; $r = 0.327$, $P = 0.028$; and $r = 0.416$, $P = 0.004$, respectively). Yet, correlations were not established between LAB viable counts and *E. coli*, TBC, TEC, M-Y, and pH.

Influence of Fermentation on Bovine Colostrum BA

Biogenic amine content in BC samples is shown in Table 3. Spermine and spermidine were not detected in BC samples, and the dominant BA were putrescine (**PUT**) and cadaverine (**CAD**). Histamine (**HIS**) was only found in nonfermented BC obtained from farm (5; 16.8 mg/kg). However, tyramine (**TYR**) was found in all samples, except for nonfermented and fermented samples obtained from farm (5). Comparing TYR content in nonfermented and fermented sample

Table 3. Average values and SE of bovine colostrum biogenic amines (BA)[1]

Sample group no.[2]	TRP, mg/kg	PHE, mg/kg	PUT, mg/kg	CAD, mg/kg	TYR, mg/kg	Total BA content, mg/kg
Control						
(1)	ND[3]	9.22 ± 0.90^{ab}	59.1 ± 2.3^{b}	52.1 ± 2.9^{b}	9.50 ± 0.91^{ab}	129.9 ± 5.5^{b}
(2)	4.64 ± 0.24^{c}	8.16 ± 0.59^{a}	79.7 ± 6.2^{c}	70.7 ± 3.3^{c}	10.4 ± 0.3^{b}	173.6 ± 8.0^{d}
(3)	ND	8.66 ± 0.26^{a}	160.6 ± 5.5^{d}	77.2 ± 5.8^{c}	17.6 ± 1.4^{c}	264.2 ± 18.7^{e}
(4)	2.59 ± 0.17^{b}	10.6 ± 0.9^{b}	76.4 ± 2.7^{c}	53.7 ± 5.1	8.20 ± 0.78^{a}	151.5 ± 12.5^{c}
(5)	1.42 ± 0.13^{a}	7.90 ± 0.60^{a}	38.1 ± 3.5^{a}	34.1 ± 2.9	ND	98.3 ± 6.2^{a}
LUHS135						
(1)	1.47 ± 0.12^{a}	8.15 ± 0.69^{bc}	104.9 ± 9.8^{b}	109.2 ± 5.2^{c}	15.8 ± 1.0^{c}	239.5 ± 11.1^{b}
(2)	6.46 ± 0.25$_{c}$	6.95 ± 0.42^{a}	103.7 ± 7.2^{b}	127.8 ± 10.7^{d}	12.8 ± 0.7^{b}	257.7 ± 9.4^{b}
(3)	3.57 ± 0.21^{b}	7.37 ± 0.40^{ab}	127.1 ± 8.0^{c}	140.4 ± 4.6^{e}	26.5 ± 1.5^{d}	305.0 ± 17.1^{c}
(4)	7.34 ± 0.23^{d}	9.14 ± 0.56^{c}	60.3 ± 4.3^{a}	41.8 ± 3.1^{a}	10.5 ± 1.0^{a}	129.1 ± 7.2^{a}
(5)	ND	9.36 ± 0.71^{c}	58.8 ± 2.6^{a}	51.5 ± 4.3^{b}	ND	119.7 ± 8.8^{a}
LUHS244						
(1)	ND	8.85 ± 0.66^{b}	127.0 ± 8.1^{c}	93.2 ± 4.3^{d}	9.00 ± 0.34^{b}	238.0 ± 21.7^{c}
(2)	ND	9.11 ± 0.73^{b}	146.3 ± 13.1^{cd}	84.3 ± 6.6^{cd}	15.4 ± 1.4^{c}	255.1 ± 11.4^{c}
(3)	ND	6.96 ± 0.57^{a}	143.4 ± 6.8^{d}	79.5 ± 4.1^{c}	16.1 ± 1.4^{c}	245.9 ± 18.8^{c}
(4)	0.650 ± 0.043^{a}	9.52 ± 0.76^{b}	60.7 ± 2.0^{a}	34.9 ± 3.0^{a}	3.80 ± 0.2^{a}	109.6 ± 5.4^{a}
(5)	1.66 ± 0.08^{b}	6.84 ± 0.52^{a}	75.6 ± 6.8^{b}	62.9 ± 2.9^{b}	ND	147.0 ± 12.2^{b}

$^{a–e}$Mean values between different samples in group (groups: nonfermented, fermented with LUHS135, and fermented with LUHS244) within a row with different letters are significantly different ($P \leq 0.05$).

[1]Paired-samples *t*-test was used to evaluate significant differences between groups of bovine colostrum samples; TRP = tryptamine; PHE = phenylethylamine; PUT = putrescine; CAD = cadaverine; TYR = tyramine.

[2](1) = colostrum from Dauksiai, Lithuania, (2) = colostrum from Sauselio village, Lithuania, (3) = colostrum from Geluvos village, Lithuania, (4) = colostrum from Paliepiu village, Lithuania, (5) = colostrum from Alksnupiu village, Lithuania; LUHS135 = fermented with *Lactiplantibacillus plantarum*; LUHS244 = fermented with *Lacticaseibacillus paracasei*.

[3]ND = not detected.

groups, concentration increased in most of the samples fermented with *Lp. plantarum*, particularly, on average, 66.3% in samples from farm (1), 23.1% in samples from farm (2), 50.6% in samples from farm (3), and 28.1% in samples from farm (4). However, different tendencies were established in samples fermented with *Lc. paracasei*: in sample groups (1) and (3) TYR content was similar to the nonfermented ones; in samples group (2) TYR content increased, on average, 48.1%; and in sample group (4) TYR content decreased, on average, 53.7%. Tryptamine (TRP) content in most of the nonfermented and fermented samples was lower than 5 mg/kg, except for the samples (2) and (4) fermented with *Lp. plantarum*—in which TYR concentration was 6.46 and 7.34 mg/kg, respectively. Phenylethylamine (PHE) was detected in all samples and, in most of cases, no significant differences were established between nonfermented and fermented samples in PHE content, except for the samples (2) and (3) fermented with *Lp. plantarum* strain and for the samples (3) fermented with *Lc. paracasei* strain, in which PHE concentration was lower, and unlike for the sample (5) fermented with *Lp. plantarum* strain, in which PHE concentration was higher. Comparing PUT concentration, in samples (1), (2), and (5) fermented with *Lp. plantarum* and *Lc. paracasei* strains PUT content was higher than in nonfermented ones (on average, 77.5%, 30.1%, 54.3%, 114.9%, 83.6%, and 98.4% higher, respectively). In comparison CAD concentration in nonfermented and fermented sample groups, higher concentration of CAD was frequently found in fermented samples, but samples (4) fermented with *Lp. plantarum* and *Lc. paracasei* strains—which showed lower CAD content in comparison with nonfermented samples. Moreover, in samples (3) fermented with *Lc. paracasei* strain CAD content was similar as in nonfermented ones (on average, 78.4 mg/kg).

Multivariate ANOVA showed that the farm (agricultural company) was a significant factor in TRP, PHE, HIS, and TYR content in BC samples ($P \leq 0.0001$, $P = 0.027$, $P = 0.002$, and $P = 0.029$, respectively). Likewise, LAB strain used for fermentation was a significant factor on HIS content in BC ($P \leq 0.01$). Similarly, interaction of analyzed factors was significant regarding TRP, PHE, HIS, TYR, and total BA content in BC samples ($P \leq 0.0001$, $P = 0.016$, $P \leq 0.0001$, $P = 0.035$ and $P = 0.026$, respectively). Also, PUT, CAD, TYR, and total BA content showed positive moderate and weak correlations with BC DM content (r = 0.455, $P = 0.002$; r = 0.495, $P \leq 0.001$; r = 0.322, $P = 0.031$; and r = 0.472, $P \leq 0.001$, respectively). Usually, during the fermentation process solids in fermentable substrate are reduced because of the microbial metabolic activities. Also, *E. coli* viable counts showed weak negative and positive correlations, respectively, with PHE and HIS contents in BC (r = −0.294, $P = 0.05$; and r = 0.304, $P = 0.043$, respectively), as well as TBC in

Table 4. Average values and SE of nonfermented bovine colostrum volatile compounds[1]

Volatile compound, % of total volatile compound	Control sample[2]				
	(1)	(2)	(3)	(4)	(5)
Alcohols					
1-Pentanol	ND[3]	5.41 ± 0.28^{b}	8.92 ± 0.62^{c}	10.7 ± 0.9^{d}	0.210 ± 0.018^{a}
3-Methyl-2-propyl-1-pentanol	1.70 ± 0.13^{b}	2.79 ± 0.18^{c}	1.22 ± 0.13^{a}	3.66 ± 0.31^{d}	3.34 ± 0.26^{d}
2-ethyl-1-Hexanol	33.3 ± 1.9^{e}	9.33 ± 0.46^{c}	19.4 ± 0.7^{d}	1.75 ± 0.164^{a}	7.43 ± 0.42^{b}
Aldehydes					
Phenylacetaldehyde	ND	1.36 ± 0.07^{b}	1.44 ± 0.04^{b}	5.11 ± 0.211^{c}	0.070 ± 0.009^{a}
Alkanes					
Decane	23.49 ± 0.86^{a}	24.1 ± 2.49^{a}	22.7 ± 1.52^{a}	31.1 ± 2.6^{b}	40.4 ± 2.86^{c}
3,6-dimethyldecane	5.74 ± 0.48^{a}	7.90 ± 0.39^{b}	6.04 ± 0.22^{a}	7.58 ± 0.725^{b}	9.26 ± 0.71^{c}
Undecane	1.62 ± 0.06^{a}	2.77 ± 0.12^{c}	3.04 ± 0.10^{d}	2.24 ± 0.13^{b}	2.85 ± 0.12^{cd}
5-(2-methylpropyl)nonane	0.410 ± 0.040^{a}	1.01 ± 0.05^{d}	0.890 ± 0.032^{c}	0.880 ± 0.076^{bc}	0.780 ± 0.044^{b}
Dodecane	13.41 ± 1.16^{a}	20.4 ± 1.8^{c}	18.3 ± 1.2^{bc}	19.0 ± 1.9^{bc}	16.3 ± 1.51^{b}
4-methyldodecane	0.580 ± 0.055^{a}	1.01 ± 0.08^{c}	0.960 ± 0.034^{c}	0.610 ± 0.035^{a}	0.730 ± 0.07^{b}
Tridecane	1.74 ± 0.09^{ab}	2.51 ± 0.10^{c}	1.85 ± 0.14^{b}	1.79 ± 0.06^{ab}	1.60 ± 0.10^{a}
Tetradecane	6.05 ± 0.22^{d}	3.59 ± 0.21^{c}	3.22 ± 0.19^{c}	2.48 ± 0.18^{b}	1.49 ± 0.13^{a}
Alkylbenzenes					
1,3-di-tert-butylbenzene	9.27 ± 0.73^{a}	13.5 ± 0.69^{b}	9.23 ± 0.74^{a}	9.83 ± 0.69^{a}	9.98 ± 0.664^{a}
2,4-di-tert-butylphenol	1.26 ± 0.05^{b}	1.82 ± 0.17^{c}	1.09 ± 0.05^{a}	1.88 ± 0.18^{c}	1.22 ± 0.08^{b}
Esters					
Ethyl octanoate	ND	ND	ND	ND	0.120 ± 0.009
Ethyldecanoate	ND	ND	ND	ND	0.060 ± 0.005
Ethyldodecanoate	ND	ND	ND	ND	0.740 ± 0.048
Organic acids					
Hexanoic acid	ND	ND	0.200 ± 0.013^{a}	ND	0.440 ± 0.032^{b}
Octanoic acid	1.48 ± 0.09^{a}	1.86 ± 0.18^{b}	1.47 ± 0.08^{a}	1.41 ± 0.09^{a}	1.61 ± 0.14^{ab}
n-Decanoic acid	ND	0.570 ± 0.041^{a}	ND	ND	0.580 ± 0.004^{a}
Dodecanoic acid	ND	ND	ND	ND	0.720 ± 0.064

$^{a–e}$Mean values between different samples in group (groups: nonfermented, fermented with LUHS135, and fermented with LUHS244) within columns with different letters are significantly different ($P \leq 0.05$).

[1]Paired–samples *t*-test was used to evaluate significant differences between groups of bovine colostrum samples.

[2](1) = colostrum from Dauksiai, Lithuania, (2) = colostrum from Sauselio village, Lithuania, (3) = colostrum from Geluvos village, Lithuania, (4) = colostrum from Paliepiu village, Lithuania, (5) = colostrum from Alksnupiu village, Lithuania; LUHS135 = fermented with *Lactiplantibacillus plantarum*; LUHS244 = fermented with *Lacticaseibacillus paracasei*.

[3]ND = not detected.

BC showed weak positive correlation with CAD content (r = 0.303, $P = 0.043$). TEC showed positive moderate correlations with CAD, HIS, TYR, and the total BA content (r = 0.318, $P = 0.033$; r = 0.343, $P = 0.021$; r = 0.302, $P = 0.044$, and r = 0.405, $P = 0.006$, respectively). The M-Y viable counts in BC showed positive correlations with CAD, HIS and total BA content (r = 0.310, $P = 0.038$; r = 0.407, $P = 0.005$; and r = 0.362, $P = 0.015$, respectively).

Influence of Fermentation on Bovine Colostrum VC

Volatile compounds (% from the total VC content) in nonfermented and fermented BC samples are shown in Tables 4 and 5, respectively. The main VC in nonfermented and fermented BC were decane, 2-ethyl-1-hexanol, dodecane, 1,3-di-tert-butylbenzene, 3,6-dimethyldecane and tetradecane. It was found that the farm (origin) and the interaction of factors (farm and LAB strain used for fermentation) were statistically significant on 2-ethyl-1-hexanol formation in BC (Table 6). Our study showed, that dominant VC in BC is similar but some of them are substantially influenced by different farms, different LAB strain used for fermentation and their interaction (Table 6). Furthermore, significant weak negative correlations were found between BC pH and 1-pentanol and phenylacetaldehyde (r = −0.389 and r = −0.299, respectively; Table 7). *E. coli* viable counts in BC exhibited significant negative weak and moderate correlations with 1-pentanol, phenylacetaldehyde, dodecane and tetradecane (r = −0.410, r = −0.498, r = −0.354, and r = −0.332, respectively). Significant positive correlations were found between TEC and 2-ethyl-1-hexanol, 3,6-dimethyldecane, undecane, 1,3-di-tert-butylbenzene, tridecane, tetradecane, 2,4-di-tert-butylphenol (r = 0.564, r = 0.321, r = 0.435, r = 0.683, r = 0.595, r = 0.821, and r = 0.518, respectively). In addition, M-Y viable counts in BC showed significant correlations with 7 VC and TBC with 5 out of 21 identified VC (Table 7).

Table 5. Average values and SE of fermented bovine colostrum volatile compounds[1,2]

	(1)		(2)		(3)		(4)		(5)	
Volatile compound	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244
Alcohols, % from the total VC content										
1-Pentanol	0.28 ± 0.014^{a}	ND[3]	ND	ND	3 ± 0.109^{c}	2.37 ± 0.09^{b}	4.62 ± 0.43^{d}	8.2 ± 0.55^{e}	12.8 ± 1.3^{f}	11.7 ± 0.4^{f}
3-Methyl-2-propyl-1-pentanol	1.67 ± 0.12^{ab}	1.84 ± 0.14^{b}	1.55 ±0.058 a	1.81 ±0.12 b	2.49 ± 0.21^{c}	2.9 ± 0.28^{cd}	3 ± 0.16^{d}	3.04 ± 0.16^{d}	3.47 ± 0.15^{e}	3.25 ± 0.17^{de}
2-ethyl-1-Hexanol	28.6 ± 2.9^{g}	20.7 ± 0.86^{f}	37.5 ±3.5^{h}	35.2 ±3.31^{h}	25.3 ± 1.17^{g}	5.19 ± 0.39^{d}	3.88 ± 0.15^{c}	3.46 ± 0.20^{b}	11.6 ± 1.1^{e}	0.33 ± 0.013^{a}
Aldehydes, % from the total VC content										
Phenylacetaldehyde	0.32 ± 0.029^{b}	0.18 ± 0.010^{a}	ND	ND	2.84 ± 0.15^{d}	2.05 ± 0.12^{c}	7.08 ± 0.67^{f}	3.98 ± 0.14^{e}	5.99 ± 0.45^{f}	3.82 ± 0.19^{e}
Alkanes, % from the total VC content										
Decane	24.6 ± 1.4^{b}	31.9 ± 2.9^{cd}	23.4 2.1^{ab}	24.8 ± 1.6^{b}	20.5 ± 1.5^{a}	40.2 ± 2.0^{e}	35.1 ± 1.2^{d}	30.1 ±1.4^{c}	28.6 ± 2.2^{c}	32.1 ± 2.4^{cd}
3,6-dimethyldecane	6.04 ± 0.56^{b}	7.12 ± 0.31^{c}	4.88 ± 0.25^{a}	7.62 ± 0.62^{cd}	7.93 ± 0.76^{cd}	6.67 ± 0.63^{bc}	6.87 ± 0.64^{bc}	7.86 ± 0.29^{d}	5.84 ± 0.30^{b}	8.02 ± 0.78^{cd}
Undecane	1.75 ± 0.14$_{a}$	1.63 ± 0.12^{a}	1.74 ± 0.09^{a}	1.83 ± 0.16^{ab}	2.28 ± 0.14^{b}	2.85 ± 0.17^{c}	2.18 ± 0.11^{b}	2.24 ± 0.16^{b}	1.82 ± 0.09^{a}	2.05 ± 0.13^{b}
5-(2-methylpropyl)nonane	0.48 ± 0.033^{ab}	0.42 ± 0.036^{a}	0.48 ± 0.020^{b}	0.76 ± 0.067^{d}	0.99 ± 0.050^{e}	0.64 ± 0.019^{c}	0.65 ± 0.025^{c}	0.95 ± 0.067^{e}	0.58 ± 0.049^{c}	0.77 ± 0.046^{d}
Dodecane	13.8 ± 0.7^{ab}	17.9 ± 1.1^{c}	13.2 ± 0.5^{a}	13.3 ± 1.3^{ab}	15.4 ± 1.1^{b}	17.6 ± 0.8^{c}	17.6 ± 1.1^{bc}	18.6 ± 0.9^{c}	15.1 ± 0.7^{b}	18.3 ± 1.17^{c}
4-Methyldodecane	0.619 ± 0.050^{b}	0.82 ± 0.065^{d}	0.33 ± 0.030^{a}	0.72 ± 0.029^{c}	0.65 ± 0.030^{b}	0.84 ± 0.048^{d}	0.85 ± 0.052^{d}	1.02 ± 0.07^{e}	0.81 ± 0.076^{cd}	0.83 ± 0.036^{d}
Tridecane	2.11 ± 0.07^{bc}	1.92 ± 0.15^{b}	2.3 ± 0.09^{c}	1.57 ± 0.08^{a}	2.34 ± 0.14^{c}	2.25 ± 0.10^{c}	2.32 ± 0.184^{c}	2.16 ± 0.12^{bc}	2.29 ± 0.19^{c}	2.05 ± 0.18^{bc}
Tetradecane	3.77 ± 0.19^{f}	3.21 ± 0.14^{e}	2.9 ± 0.19^{e}	2.07 ± 0.08^{bc}	2.55 ± 0.08^{d}	2.93 ± 0.25^{e}	2.25 ± 0.16^{c}	2.56 ± 0.09^{d}	1.63 ± 0.07^{a}	1.89 ± 0.18^{b}
Alkylbenzenes, % from the total VC content										
1,3-di-tert-butylbenzene	12.4 ± 1.2^{c}	9.37 ± 0.88^{b}	7.53 ± 0.53^{a}	8.02 ± 0.54^{ab}	10.8 ± 0.47^{c}	11.2 ± 1.02^{bc}	10.4 ± 0.8^{bc}	9.67 ± 0.54^{b}	7.98 ± 0.64^{ab}	12.1 ± 1.0^{c}
2,4-di-tert-butylphenol	2.55 ± 0.22^{f}	1.45 ± 0.07^{c}	1.72 ± 0.13^{de}	1.25 ± 0.13^{b}	0.96 ± 0.071^{a}	1.6 ± 0.06^{d}	1.79 ± 0.05^{e}	1.36 ± 0.08^{bc}	1.38 ± 0.13^{bc}	1.42 ± 0.05^{bc}
Esters, % from the total VC content										
Ethyl octanoate	ND[3]	ND	ND	ND	0.02 ± 0.001^{a}	ND	ND	3.21 ± 0.22^{b}	ND	ND
Ethyldecanoate	ND	ND	ND	0.02 ± 0.002^{a}	0.02 ± 0.001^{a}	ND	ND	ND	ND	ND
Ethyldodecanoate	0.33 ± 0.012^{b}	ND	ND	ND	0.07 ± 0.001^{a}	ND	ND	ND	ND	ND
Organic acids, % from the total VC content										
Hexanoic acid	ND	ND	ND	ND	ND	0.01 ± 0.001	ND	0.01 ± 0.002	ND	ND
Octanoic acid	0.73 ± 0.047^{b}	1.61 ± 0.11^{d}	2.48 ± 0.22^{f}	1.09 ± 0.04^{c}	2.02 ± 0.14^{e}	0.55 ± 0.027^{a}	1.5 ± 0.06^{d}	1.56 ± 0.14^{d}	ND	1.47 ± 0.11^{d}

Continued

Table 5 (Continued). Average values and SE of fermented bovine colostrum volatile compounds[1,2]

Volatile compound	(1)		(2)		(3)		(4)		(5)	
	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244	LUHS135	LUHS244
n-Decanoic acid	ND	ND	ND	ND	ND	0.09 ± 0.005	ND	ND	ND	ND
Dodecanoic acid	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a–h]Mean values between different samples in group (groups: nonfermented, fermented with LUHS135, and fermented with LUHS244) within columns with different letters are significantly different ($P \leq 0.05$).

[1]Paired–samples *t*-test was used to evaluate significant differences between groups of bovine colostrum samples.

[2](1) = colostrum from Dauksiai, Lithuania, (2) = colostrum from Sauselio village, Lithuania, (3) = colostrum from Geluvos village, Lithuania, (4) = colostrum from Paliepiu village, Lithuania, (5) = colostrum from Alksnupiu village, Lithuania; LUHS135 = fermented with *Lactiplantibacillus plantarum*; LUHS244 = fermented with *Lacticaseibacillus paracasei*.

[3]ND = not detected.

DISCUSSION

Despite, that no significant differences were observed in pH and DM of nonfermented samples, it was reported, that, usually, the pH of BC can vary from 5.59 to 6.42 (Stewart et al., 2005; Cummins et al., 2017; Hyrslova et al., 2020). In comparison nonfermented and fermented BC samples, in the latter pH average values decreased. Our previous studies disclosed that *Lp. plantarum* and *Lc. paracasei* strains possess versatile metabolism of carbohydrates, including very good lactose conversion (Bartkiene et al., 2020a). Moreover, their survival at low pH (pH of 2.5 for 2 h) was 5.72 and 7.69 $\log_{10}$ cfu/mL for *Lp. plantarum* and *Lc. paracasei*, respectively (Bartkiene et al., 2020a). The main carbohydrates in BC are lactose, oligosaccharides, glycolipids, glycoproteins and nucleotide sugars (Playford and Weiser, 2021). Lastly, based on these results one may conclude that BC is a suitable substrate for the fermentation with selected LAB strains.

Analyzing color characteristics of nonfermented BC sample groups, no significant differences between L* (lightness) and b* (yellowness) coordinates were found. However, the origin (farm) of colostrum, LAB strain used for fermentation and the interaction of these factors were statistically significant on BC a* coordinate. It was stated that color of BC reflects its quality (El-Hatmi et al., 2023), and the reddish color of milk can be related to udder infections or teat injuries (Brandt et al., 2010). Gross et al. (Gross et al., 2014) reported that color parameters clearly correlate with BC composition (IgG, fat, protein, and lactose) and a negative correlation between BC L* values and protein content was established. El-Hatmi et al. (2023) reported a strong positive correlation between protein content (%) and DM (%) with a* values of camel colostrum, as well as a slight correlation between ash content and a* coordinates. However, the data about the changes of colostrum chromaticity parameters during the fermentation process are scarce. Observing microbiological parameters of nonfermented BC, no significant differences were found in samples collected from different farms. However, the colostrum contamination could depend on many factors, including hygiene and cow lactation period (Stewart et al., 2005). It was reported, that the LAB count in BC varied from 4.5 to 5.1 ($\log_{10}$ cfu/mL), the total bacteria count from 3.60 to 7.26 ($\log_{10}$ cfu/mL), the total coliform count from 3.39 to 6.39 ($\log_{10}$ cfu/mL), and enterobacteria count from 1.4 ± 0.59 to 3.5 ± 0.55 ($\log_{10}$ cfu/mL; Santos et al., 2017). Other studies showed a wide range of total microorganisms count in BC, ranging from 1.4 to 7.0 $\log_{10}$ cfu/mL (Gelsinger et al., 2015), from 3.0 to 6.8 $\log_{10}$ cfu/mL (Morrill et al., 2012), from 5.4 to 7.2 log cfu/mL (Dunn

Table 6. Significance of the influence of the factors (farm/agricultural company and lactic acid bacteria [LAB] strain used for fermentation) and their interaction on the formation of volatile compounds in bovine colostrum

Volatile compound	Significance (P) of the influence of factors and their interaction*		
	Factor farm	Factor × LAB strain used for fermentation	Interaction of the factors × farm vs. LAB strain used for fermentation
Alcohols			
1-Pentanol	0.004	0.87	0.034
3-Methyl-2-propyl-1-pentanol	0.152	0.457	0.482
2-ethyl-1-Hexanol	0.016	0.72	0.022
Aldehydes			
Phenylacetaldehyde	0.0001	0.116	0.333
Alkanes			
Decane	0.247	0.422	0.972
3,6-dimethyldecane	0.652	0.843	0.933
Undecane	0.572	0.949	0.808
5-(2-methylpropyl)nonane	0.304	0.127	0.553
Dodecane	0.329	0.964	0.869
4-methyldodecane	0.687	0.056	0.596
Tridecane	0.998	0.779	0.998
Tetradecane	0.189	0.544	0.968
Alkylbenzenes			
1,3-di-tert-butylbenzene	0.748	0.624	0.912
2,4-di-tert-butylphenol	0.413	0.939	0.406
Esters			
Ethyl octanoate	0.003	0.012	0.0001
Ethyldecanoate	0.016	0.163	0.0001
Ethyldodecanoate	0.009	0.049	0.0001
Organic acids			
Hexanoic acid	0.009	0.001	0.002
Octanoic acid	0.282	0.495	0.292
n-Decanoic acid	0.016	0.001	0.002
Dodecanoic acid	0.002	0.011	0.0001

*Factors or factors interaction are significant when $P \leq 0.05$.

et al., 2017), from 1.86 to 11.02 $\log_{10}$ cfu/mL (Swan et al., 2007). In our study observed a slightly higher contamination, in comparison, with previous studies. However, different trends were established in fermented BC samples, and, LAB strain used for fermentation and factor's interaction (LAB strain used for fermentation and farm) were significant on *E. coli* viable counts in BC. Microbial contamination of BC is associated with the nonhygienic udder, milking and feeding equipment, as well as storage (Donahue et al., 2012; Godden et al., 2019). In our study obtained results, for the nontreated colostrum, are slightly different with those of Santos et al., who found that the LAB, enterobacteria, and yeast counts of BC from 3 different farms were 4.5 to 5.1, 1.4 to 3.5, and 1.3 to 1.7 $\log_{10}$ cfu/mL, respectively (Santos et al., 2017). The differences, reported by different studies, may be explained by variances in geography, climate, technology, management practices, and different hygiene condition in farms (Šlosárková et al., 2021). Also, the LAB count in colostrum can rapidly increase, due to storage conditions (Santos et al., 2017). The enterobacteria numbers are usually related to the lack of hygiene before, during, and after colostrum milking (Santos et al., 2017). However, fermentation with selected LAB, which possesses antimicrobial properties, allows for the biological preservation of BC (Fasse et al., 2021). The synthesis of numerous antibacterial substances by LAB is well-known (Mörschbächer and Granada, 2022), and, the reduced contamination of fermented BC could be associated with the ability of LAB to produce inhibitory compounds (organic acids, hydrogen peroxide, antimicrobial peptides, and so on (Saalfeld et al., 2016). The high antimicrobial activity of *Lacticaseibacillus paracasei* LUHS244 was already reported in our previous research (Bartkiene et al., 2020a). Our study showed that microbial contamination of BC was similar in all the tested farms. Nevertheless, different LAB strains used for fermentation showed different capacities toward microbial contamination reduction and, in this regard, *Lc. paracasei* was more effective in comparison with *Lp. plantarum* strain. Furthermore, no correlations were found between LAB viable counts and microbial contamination, despite the fact that pH reduction (by LAB) was the most important factor desirably contributing toward BC contamination reduction. Aside from the ability of microbiological strains to ferment a wide range of carbohydrates, variation in the lactose content (as a primary source for LAB

Table 7. Correlations and their significance between bovine colostrum volatile compounds and bovine colostrum pH and microbiological parameters[1]

Volatile compound	pH	LAB	*Escherichia coli*	TBC	TEC	M-Y
Alcohols						
1-Pentanol	−0.389**	−0.036	−0.410**	−0.283	−0.118	−0.227
3-Methyl-2-propyl-1-pentanol	−0.097	0.148	−0.345*	−0.078	0.101	0.079
2-ethyl-1-Hexanol	0.258	−0.277	0.285	0.374*	0.564**	0.537**
Aldehydes						
Phenylacetaldehyde	−0.299*	0.122	−0.498**	−0.252	−0.193	−0.315*
Alkanes						
Decane	−0.072	−0.063	−0.237	0.038	0.217	0.147
3,6-dimethyldecane	−0.063	−0.074	−0.26	0.106	0.321*	0.191
Undecane	0.096	−0.168	−0.126	0.167	0.435**	0.385**
5-(2-methylpropyl)nonane	−0.179	−0.17	−0.262	0.019	0.167	0.073
Dodecane	−0.156	−0.105	−0.354*	−0.023	0.176	0.059
4-methyldodecane	−0.132	−0.157	−0.225	0.058	0.2	0.146
Tridecane	0.048	−0.052	−0.032	0.319*	0.595**	0.505**
Tetradecane	0.237	−0.123	0.332*	0.560**	0.821**	0.779**
Alkylbenzenes						
1,3-di-tert-butylbenzene	0.243	−0.079	0.101	0.421**	0.683**	0.590**
2,4-di-tert-butylphenol	0.09	−0.087	0.055	0.327*	0.518**	0.479**
Esters						
Ethyl octanoate	0.015	0.069	−0.275	−0.202	−0.218	−0.159
Ethyldecanoate	−0.013	−0.02	−0.254	−0.163	−0.111	−0.125
Ethyldodecanoate	0.075	0.08	−0.094	−0.063	−0.015	0.048
Organic acids						
Hexanoic acid	−0.009	−0.139	−0.207	−0.214	−0.117	−0.076
Octanoic acid	−0.188	−0.112	−0.183	0.081	0.262	0.142
n-Decanoic acid	0.151	0.065	0.039	0.071	0.163	0.184
Dodecanoic acid	−0.012	0.072	−0.209	−0.193	−0.167	−0.106

[1]LAB = lactic acid bacteria; TBC = total bacteria viable counts; TEC = total Enterobacteriaceae viable counts, M-Y = mold and yeast viable counts; r = Pearson Correlation.

**Correlation is significant at the 0.01 level (2-tailed); *Correlation is significant at the level of 0.05 (2-tailed).

metabolism and organic acid production) of BC can be a critical factor in ensuring its effective preservation when fermentation is used. Additionally, the survival of bacteria in the substrate and their resistance to survive at low pH values can lead to higher acid productions and a more effective preservation of BC. Our previous studies showed that *Lc. paracasei* tolerance to low pH conditions is much higher in comparison with *Lp. plantarum* strain (after 2 h at pH 2.5 the viable counts were 9.29 $\log_{10}$ cfu/mL for *Lc. paracasei* and 5.72 $\log_{10}$ cfu/mL for *Lp. Plantarum*; Bartkiene et al., 2020a).

Despite, that LAB are generally recognized as safe (GRAS) microorganisms, they could be related to BA formation in fermentable substrate. Also, our study showed, that the farm was a significant factor in TRP, PHE, HIS and TYR content in BC samples, as well as LAB strain used for fermentation was a significant factor on HIS content in BC, and interaction of analyzed factors was significant regarding TRP, PHE, HIS, TYR, and total BA content in BC samples. It was reported that postbiotics produced by LAB can reduce the production of BA by foodborne pathogens (i.e., *Salmonella paratyphi* A and *E. coli*; Yilmaz et al., 2022). However, despite that LAB are GRAS, they are also able to convert amino acids into BA via decarboxylase activity during fermentation processes of different substrates (Özogul and Hamed, 2018). In the current research study, correlations between LAB viable counts and BA concentration were not found, and such a behavior can be explained by the fact that LAB viable counts is not always related to the decarboxylase activity and the concomitant BA excreted to the substrate medium. As a matter of fact, most of the samples fermented with LAB showed higher BA content in comparison with nonfermented ones. The concentration and type of BA formed is intimately related to the nature of substrate and the kind of microorganisms (Gunasekaran et al., 2020). It was reported that among 66 LAB strains tested, 21 lactobacilli and all 16 enterococci were BA producers, and TYR was the main BA in addition to PHE, TRP, PUT and CAD; however, none of the LAB produced histamine (Bover-Cid et al., 2001). Likewise, the composition of amino acids, as the main precursors for BA formation, should be taken into consideration during colostrum fermentation, because the profile of amino acids could vary as according to the animal feeding, which, concomitantly, may lead to substantial differences in BA formation throughout fermentation. Finally, from above one should emphasize, that despite fermentation

with LAB is usually described as a safe and natural process with many technological advantages, control of BA in the end product is crucial.

Experimental data on BC VC are scarce. The milk's flavor is highly affected by the type of feed and its metabolism by ruminants. Important precursors that contributes to the milk's flavor are mainly carboxylic acids, alcohols, aldehydes, ketones, free fatty acids and sulfur compounds (Biçer et al., 2021). The main VC in nonfermented and fermented BC were decane, 2-ethyl-1-hexanol, dodecane, 1,3-di-tert-butylbenzene, 3,6-dimethyldecane and tetradecane. It was reported that decane and dodecane are abundantly present in milk from sheep of Assaf breed, and a negative correlation exists in sheep milk between *Lactobacillus* (detected at the highest level in all sheep groups) and decane and dodecane (Biçer et al., 2021). 2-Ethyl-1-hexanol odor is described as citrus, fresh, floral, oily and sweet. This VC is an aroma component of plants (e.g., wheat and laminaceae; Birkett et al., 2004; Li et al., 2014); it was also identified in the breath of cattle (Ellis et al., 2014). Regarding 1,3-di-tert-butylbenzene, it was conveyed that this is a product of radiolysis of the antioxidant Irgafos used in packaging (Jeon et al., 2007). This finding can be explained by the fact that BC was kept at freezing temperatures in plastic bags, which allows the migration of various compounds from the package to the colostrum (Velickova Nikova et al., 2022). Additionally, 1,3-di-tert-butylbenzene can be related to the contamination of plants by fungi (*Metarhizium brunneum*; Cotes et al., 2015). In our study, analysis of VC of feed were not included, however, taking into consideration, that 1,3-di-tert-butylbenzene was found in colostrum samples, further analysis of feed would be prospective. 3,6-Dimethyldecane is a VC typically detected in the gas emissions from vehicles (Huang et al., 2020). Additionally, 3,6-dimethyldecane as well as tetradecane were identified as compounds from polypropylene packaging (Paiva et al., 2021). Above mentioned VC, identified in colostrum samples, lead to further suggestions to analyze more factors (environment and packaging material), which could be associated with different compounds migration to BC. This study showed worrying trends about the frozen colostrum storage, because most of the predominant VC in BC were not typical for milk, as well as some of it were related to packaging material compounds. Further studies are needed to indicate origin of the identified VC in BC. The colostrum contains a variety of different macro- and microconstituents, including carbohydrates, lipids, minerals, vitamins, proteins, immunoreactive components, hormones, enzymes, diverse microbial community, and so on (Abdelsattar et al., 2022). All this complex biological system when in an unstable state (*e.g.*, nonfrozen or nondried) can change with the formation of VC. Additionally, formation of different VC is closely related to the specificity of microbial, fatty acid, AA, and sugar profiles, among others. Important to underline that these promising results show that individual VC in BC can act as biological and chemical contamination markers.

CONCLUSIONS

Nonfermented BC sample groups showed similar values of pH, DM, lightness, yellowness and microbial contamination. Fermentation with *Lactiplantibacillus plantarum* and *Lacticaseibacillus paracasei* decreased pH values, on average, to 4.40 and 4.37, respectively. The farm, LAB strain used for fermentation and interactions between these factors significantly influenced BC redness. Compared with *Lp. plantarum*, *Lc. paracasei* was more effective toward microbial contamination reduction. Predominant BA in BC were putrescine and cadaverine, whereas the main VC found in nonfermented and fermented BC were decane, 2-ethyl-1-hexanol, dodecane, 1,3-di-tert-butylbenzene, 3,6-dimethyldecane and tetradecane. This study showed worrying trends about the frozen colostrum storage, because most of the predominant VC in BC were not typical for milk, as well as some of it were related to packaging material compounds. Further studies are needed to indicate origin of the identified VC in BC. Significant correlations between individual VC and microbial contamination were established. Our findings demonstrated that different VC in BC can serve as markers of both biological and chemical contaminations. Finally, it should be noted that although fermentation with LAB is typically characterized as a secure and natural method and with numerous benefits, monitoring of BA in the final product is highly advisable.

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